Mol Breeding (2011) 27:137–156
DOI 10.1007/s11032-010-9417-1

Development and bin mapping of strawberry genic-SSRs in diploid *Fragaria* and their transferability across the Rosoideae subfamily

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Received: 4 October 2009 / Accepted: 17 February 2010 / Published online: 20 March 2010

Abstract Cultivated strawberry (*Fragaria* × *ananassa*) together with other economically important genera such as *Rosa* (roses) and *Rubus* (raspberry and blackberry) belongs to the subfamily Rosoideae. There is increasing interest in the development of transferable markers to allow genome comparisons within the Rosaceae family. In this report, 122 new genic microsatellite (SSR) markers have been developed from cultivated strawberry and its diploid ancestor *Fragaria vesca*. More than 77% of the sequences from which the markers were developed show significant homology to known or predicted proteins and more than 92% were polymorphic among strawberry cultivars, representing valuable markers in transcribed regions of the genome. Sixty-three SSRs were polymorphic in the diploid *Fragaria* reference population and were bin-mapped together with another five previously reported but unmapped markers. In total, 72 loci were distributed across the seven linkage groups. In addition, the transferability of 174 *Fragaria* SSRs to the related *Rosa* and *Rubus* genera was investigated, ranging from 28.7% for genic-SSRs in rose to 16.1% for genomic-SSRs in raspberry. Among these markers, 33 and 16 were both localized in the diploid *Fragaria* reference map and cross-amplified in rose and raspberry, respectively. These results indicate that transferability of SSRs across the Rosoideae subfamily is limited. However, we have identified a set of *Fragaria* markers, polymorphic in the diploid reference population, which cross-amplified in both *Rosa* and *Rubus*, which represents a valuable tool for comparative mapping and genetic diversity analyses within the Rosoideae subfamily.

Keywords Comparative mapping · Synteny · Strawberry · Rose · Raspberry · Rosoideae

Electronic supplementary material The online version of this article (doi:10.1007/s11032-010-9417-1) contains supplementary material, which is available to authorized users.

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Introduction

The genus *Fragaria* belongs to the Rosaceae family and comprises 23 species, including the commercially important strawberry (*Fragaria* × *ananassa*) (Rousseau-Gueutin et al. 2009). The genus displays a series of ploidy levels, ranging from diploid species such as *Fragaria vesca* ($2n = 2x = 14$) to decaploid species such as some accessions of *Fragaria iturupensis* ($2n = 10x = 70$). The cultivated strawberry, *F.* × *ananassa,* is an octoploid ($2n = 8x = 56$) and has been the focus of an increasing number of molecular and genetic studies in recent years (Davis et al. 2007; Folta and Davis 2006; Hokanson and Maas 2001). Molecular markers in strawberry have been developed and used to characterize germplasm collections (Davis et al. 2006; Gil-Ariza et al. 2009; Govan et al. 2008) and for genetic mapping (Cekic et al. 2001; Deng and Davis 2001; Haymes et al. 1997; Lerceteau-Kohler et al. 2004). However, most of the genetic studies have been performed in *F. vesca,* mainly due to its small genome, comparable to that of *Arabidopsis thaliana* (Akiyama et al. 2001). Furthermore, recent data indicate that an ancestor of *F. vesca* was the maternal donor of the octoploid strawberry genome, suggesting a model for the octoploid genome constitution of Y1Y1Y1′Y1′ZZZZ, in which the Y1 and Z genomes would have been donated from *F. vesca* and *F. iinumae* ancestors, respectively, (Rousseau-Gueutin et al. 2009; Shulaev et al. 2008). In addition, growing evidence points to a highly disomic behavior in the commercial octoploid strawberry, implying a less complex genome than previously anticipated (Rousseau-Gueutin et al. 2008; Sargent et al. 2009a).

In species with complex genomes, simple sequence repeats (SSRs) are the markers of choice in genetic and breeding studies due to their high polymorphism and reproducibility, multi-allelic nature and codominant inheritance. They have also proven to be very useful both for integrating mapping data and comparing maps within and across closely related genera (Gisbert et al. 2009; Pierantoni et al. 2004; Silfverberg-Dilworth et al. 2006). Several studies have shown a high level of transferability within the *Fragaria* genus (Bassil et al. 2006a, b; Davis et al. 2006; Gil-Ariza et al. 2006; Monfort et al. 2006). Genic-SSRs have been defined as SSRs derived from genes and expressed sequence tags (ESTs) representing functional markers in transcribed genes. This is likely the reason for their higher transferability across related genera (Arnold et al. 2002; Kuleung et al. 2004). These and other studies suggested that genic-SSRs are good candidates for the development of conserved orthologous markers for genetic analysis across related genera (Varshney et al. 2005).

The first genetic map of *F.* × *ananassa* was developed using amplified fragment length polymorphism (AFLP) markers (Lerceteau-Kohler et al. 2003). Although this map has recently been updated using SSRs, additional markers are needed in order to saturate the genome of this important crop (Rousseau-Gueutin et al. 2008). Very recently, another genetic linkage map has been developed for *F.* × *ananassa* predominantly using SSRs already mapped in diploid *Fragaria* (Sargent et al. 2009a). The first *F. vesca* map was developed using isoenzymes and randomly amplified polymorphic DNA (RAPD) markers (Davis and Yu 1997). Later, a transferable linkage map was developed from an interspecific cross between *F. nubicola* (recently reclassified as *F. bucharica*; Rousseau-Gueutin et al. 2008) and *F. vesca* (Sargent et al. 2004). This map has emerged as the reference map for this genus and currently contains approximately 340 SSR and gene-specific markers (Sargent et al. 2008, 2006, 2007; Vilanova et al. 2008).

Bin mapping has proven to be a robust and efficient approach for genetic mapping (Fernandez-Silva et al. 2008; Howad et al. 2005; Vision et al. 2000). This strategy improves the efficiency in saturating genetic maps with molecular markers by reducing the size of the population. Thus, new markers are added to an existing linkage map at lower resolution by genotyping only a subset of selected, highly informative genotypes (the bin set). This strategy has recently been developed for diploid *F. vesca* and a bin set of only eight plants are genotyped for an approximate mapping (Sargent et al. 2008), allowing markers or candidate genes to be time- and cost-effectively located in approximate positions on the diploid *Fragaria* reference map. In this way, a number of evenly distributed markers, or those located at positions of interest, can be selected and localized with higher accuracy using large populations of the octoploid strawberry.

The Rosaceae family, comprising over 90 genera and 3,000 species, has been recently reorganized into

three subfamilies: Dryadoideae, Spiraeoideae and Rosoideae (Potter et al. 2007). The majority of economically important crops belong to the two latter subfamilies, with genera such as *Prunus*, *Malus* and *Pyrus* in the Spiraeoideae and genera *Fragaria*, *Rosa* and *Rubus* in the Rosoideae subfamily. Comparative mapping within many plant families has been well studied: for example, in Brassicaceae, Leguminosae, Poaceae or Solanaceae (Devos and Gale 2000; Doganlar et al. 2002; Kalo et al. 2004; Lukens et al. 2003). These studies allowed the identification of a marker framework for map-based prediction of the location of candidate genes involved in agriculturally important traits within different species of the same family. Recently, efforts have been undertaken for genome comparisons within the Rosaceae (Shulaev et al. 2008). A number of reports have analyzed the transferability of SSRs and other markers across Rosaceae genera (Decroocq et al. 2003; Gasic et al. 2009; Gisbert et al. 2009; Sargent et al. 2009b, 2007; Yamamoto et al. 2004). The development and mapping of transferable markers has allowed the implementation of synteny studies within the family. Comparisons between the closely related genomes of *Malus* and *Pyrus* have shown a high level of synteny, with no major chromosomal rearrangements (Pierantoni et al. 2004; Yamamoto et al. 2004). Partial genome comparison between *Malus* and *Prunus*, which are further apart phylogenetically, has revealed regions with a high degree of synteny as well as large-scale chromosomal rearrangements (Dirlewanger et al. 2004). The genomes of the distantly related *Prunus* and *Fragaria* genera have recently been compared using 71 common markers, revealing low colinearity between these species (Vilanova et al. 2008). However, to our knowledge, no published work has yet compared the genomes of strawberry, rose and/or raspberry, all members of the subfamily Rosoideae and all of them with a basic chromosomal number of 7 ($2n = 2x = 14$).

Initial comparative genetic mapping between octoploid and diploid *Fragaria* species has revealed high levels of macrosynteny and colinearity between their genomes (Rousseau-Gueutin et al. 2008). Diploid *Fragaria* maps thus represent a reference for the genus and might provide a reduced framework of markers that could be localized in other species such as the octoploid strawberry. However, due to the octoploid nature of cultivated strawberry, the number of transferable markers available for this species is still limited for comprehensive comparative genetic analyses. The analysis of transferability of markers across genera in the Rosoideae subfamily would identify a set of anchor markers for comparative mapping. In order to increase the number of available strawberry SSRs, we have developed 122 new *Fragaria* SSR markers, most of them from a *F.* × *ananassa* fruit EST collection (Bombarely et al. submitted) but some of them also from publicly available *F. vesca* sequences. All these SSRs were evaluated for polymorphism between the parental lines of the reference diploid *Fragaria* mapping population and localized in the map. In order to identify common Rosoideae markers, we analyzed the transferability of these new SSRs together with 52 other *Fragaria* SSRs to the closely related *Rose* and *Rubus* genera. Overall, the transferability of SSR markers was limited within the Rosoideae subfamily, although a significantly higher cross-amplification was found among genic-SSRs than among SSRs developed arbitrarily from SSR-enriched genomic DNA libraries.

Materials and methods

Plant material and DNA extraction

A total of 14 strawberry cultivars was used to estimate the polymorphism of the newly developed SSR markers in *F.* × *ananassa* (Table 1). These included Chandler and Carisma as source of the majority of EST-SSRs, IFAPA selections 232 and 1,392, and cultivars Parker, Endurance and Selva as parental lines of different strawberry mapping populations and cultivars Festival, Cuesta, Solut, Josif Mahomed, Camarosa, Fern and Toyonoka covering further genetic variation. For each primer pair at least six different accessions were used in the polymorphism evaluation.

To evaluate the amplification and polymorphism in diploid *Fragaria*, up to 8 accessions were analyzed (Table 1). Genomic DNA from all *Fragaria* accessions was extracted from young leaf tissue using the DNeasy plant miniprep kit (Qiagen) according to the manufacturer's protocol.

To analyze the transferability and polymorphism in rose, we evaluated two species, the hybrid *Rosa*

Table 1 Plant material used for marker polymorphism, mapping and cross-species transferability

Species	Primary denomination/accession no.[a]	Origin
Fragaria		
F. × *ananassa*	Chandler	USA
	Carisma	Spain
	232	Spain
	1392	Spain
	Parker	USA
	Endurance	USA
	Selva	USA
	Festival	USA
	Cuesta	USA
	Salut	Poland
	Josif Mahomed	Russia
	Camarosa	USA
	Fern	USA
	Tonoyoka	Japan
F. vesca	*F. vesca* spp. *vesca* f. semperflorens/FDP 815	N.A.
	IFAPA 14	Spain
	F. vesca ssp. *bracteata*/IFAPA 184/= PI 551514	USA
	Reine des vallées/IFAPA 660	France
	F. vesca spp. *vesca* f. alba/IFAPA 596	Italy
F. bucharica	FDP 601	Pakistan
F. iinumae	PI 637963	Japan
	PI 637966	Japan
Rosa		
R. moschata × *R. chinensis*	Blush Noisette/D10	USA
R. wichurana	Basye's Thornless/E15	China
Rubus		
R. idaeus	Tulameen	Canada
	Miraz	Russia

[a] Source of accession numbers: FDP, East Malling research, UK; IFAPA, IFAPA-Centro de Churriana, Spain; PI, National clonal germplasm repository corvallis, USA; D10 and E15, IFAPA-Alameda del Obispo

moschata × *Rosa chinensis* Blush Noisette (D10) and *Rosa wichurana* (E15). These species are the parental lines of the F_1 mapping population analyzed by Dugo et al. (2005; Table 1). DNA was isolated according to Torres et al. (1993), replacing the extraction buffer by one adapted to woody species (Cheng et al. 1997).

To evaluate the transferability of *Fragaria* SSRs to raspberry (*Rubus idaeus*), young leaves were collected from cultivars Tulameen and Miraz growing in pots at the IFAPA-Centro de Churriana germplasm collection (Table 1). DNA was extracted using a modified CTAB method based on that of Doyle and Doyle (1990).

SSR selection and analysis

The new *Fragaria* SSR markers developed in this study were obtained from two different sources. The *F.* × *ananassa* EST-SSRs were designed from the FREST strawberry database, comprising ESTs from cDNA libraries of different developmental stages of strawberry fruit (Bombareli et al. submitted). Approximately 10,000 of these ESTs were grouped as either contigs or singletons and analyzed for the presence of SSR motifs using the PERL5 script MISA (Thiel et al. 2003). The *F. vesca* SSRs were obtained from public sequences in the EMBL database. A total of 150 flanking primers were designed

using the PRIMER3 program (Rozen and Skaletsky 2000). Primers were designed to have an annealing temperature (T_a) of 60–62°C, a length of 20–22 bases and generate product sizes ranging from 100 to 250 bp in length. Functional annotation of ESTs was performed using BLAST and only homologies below an E-value of 1e-10 were considered significant.

SSR amplifications were performed in a BioRad iCycler in a final volume of 15 µl, containing 1 × PCR buffer (GeneCraft; 16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl pH 8.8, 0.1% Tween 20), 2 mM $MgCl_2$, 200 µM each dNTPs, 0.2 µM of each specific primer, 0.5 U of Taq DNA polymerase (GeneCraft) and 25 ng of genomic DNA. The adopted PCR profile was as follows: 94°C for 3 min, then 35 cycles of 94°C for 30 s, annealing at 57–65°C for 30 s and extension at 72°C for 45 s. A final extension was carried out at 72°C for 3 min.

Initially, SSR validation and annealing temperature was analyzed using template DNA from *F.* × *ananassa* cv. Chandler or *F. vesca* 815 and a gradient PCR with T_a ranging from 55 to 65°C. PCR products were separated in 1.5% agarose gels and stained with ethidium bromide. Those primer pairs showing clear products of approximately the expected size were selected and used for further analyses. When primer pairs failed to amplify or the amplification product was weak, a modified Taguchi method was applied in order to optimise the conditions (Cobb and Clarkson 1994). The reaction components evaluated in the orthogonal array were the amount of DNA template, primers, dNTPs and $MgCl_2$. Each component was used at three predetermined levels, each of which occurred an equal number of times within the orthogonal array. This resulted in only nine different reaction combinations that were set up in duplicate. After separation of PCR products in 1.5% agarose and staining with ethidium bromide, an image was obtained using a Gel Doc EQ system (BioRad) and the relative band intensities were estimated using the Quantity one software (BioRad). Average band intensities for each of the nine reactions were subsequently used to determine the different component effects and to estimate the optimum reaction conditions according to the method of Cobb and Clarkson (1994). Optimized PCR conditions for primer pairs with initial weak product amplification are shown in supplementary Table 1S.

For polymorphism evaluation and mapping, PCR products were mixed with 10 µL of formamide loading buffer (98% formamide, 10 mM EDTA and 0.1% xylene cyanol), denatured at 95°C for 3 min and immediately cooled on ice. Two to four microlitres of each sample were separated by electrophoresis in 4.5% denaturing polyacrylamide gels at a constant power of 95 W for 1–3 h. The fragments were visualized by silver staining according to the protocol of Benbouza et al. (2006). Allele sizes were determined by comparison to the 10-bp DNA ladder (Invitrogen, San Diego, CA, USA).

The number of visible alleles per cultivar and the total number of alleles were evaluated by visual inspection in the analyzed cultivars. The effect of SSR type and length on the number of alleles was analyzed using the STATISTICA 7.0 software (StatSoft, Inc. 2007). A *t*-test was used to compare the mean number of alleles of polymorphic di- and trinucleotides. The correlation between SSR length and total number of alleles was studied by linear regression analysis, both globally and separately for di- and trinucleotide repeats.

Bin mapping in the diploid *Fragaria* reference map (FV × FB)

The *F. vesca* 815 parental line, the F_1 hybrid and the six bin set seedlings were genotyped with the novel polymorphic markers as described in Sargent et al. (2008). In addition, we found polymorphisms in five public SSR markers not previously mapped in the diploid reference map. These markers were ChFaM004, ChFaM005 and ChFaM007 from Gil-Ariza et al. (2006), Fvi9 developed from *F. virginiana* (Ashley et al. 2003) and PBCESSRFXA12 reported by Keniry et al. (2006). Markers were assigned to bins by visually matching the joint genotype obtained to those defining the different *Fragaria* bins. An additional seedling (31) was genotyped when the marker was assigned to the redundant bins II:50 and III:53. Gel bands were scored by two different observers. Markers that did not correspond to any of the described bins were subsequently scored in the entire FV × FB population (94 seedlings) to assign a precise position to both the marker and the novel bin. In this case, linkage analysis and map construction was performed as described by Sargent et al. (2006). Dominant markers, which have a joint genotype matching several bins, were mapped in additional individuals in order to be assigned to single bins.

SSR amplification in *Rosa* and *Rubus*

Newly developed genic-SSRs together with SSRs already developed from *Fragaria* were used to amplify DNA from the hybrid *Rosa moschata* × *Rosa chinensis* Blush Noisette (accession D10) and *Rosa wichurana* (accession E15), the parents of the rose mapping population analyzed by Dugo et al. (2005) and two accessions of raspberry, Tulameen and Miraz. Amplification reactions were carried out identically to those for *Fragaria*, the only exception being to decrease to 60°C the T_a of primer pairs when it was above that value. Amplification and polymorphism was evaluated in polyacrylamide gels and silver staining as described above.

Results

Development of novel *Fragaria* SSR markers

Recently, the number of available *F.* × *ananassa* DNA sequences has increased and around 10,000 genes and ESTs from strawberry are available in public databases (Bombarely et al., submitted; http://www.fresa.uco.uma.es). A search for SSR motifs within these sequences allowed the identification of 383 SSRs in 329 of the 7,096 unigenes analyzed. Subtracting sequences in which SSR markers had been already developed and sequences in which the SSR motifs were too close to the end, 187 sequences could be used for marker development. A total of 150 primer pairs, originating from either contigs or singletons, were designed. One hundred and forty-eight primer pairs were designed from ESTs of *F.* × *ananassa* fruit libraries and *F. vesca* EST sequences available in the Genbank database while only two (ChFaM148 and ChFvM125) were designed from genomic sequences within and adjacent to a gene respectively, thus representing markers of putative functional genes. These primers were initially tested in the genomic DNA of *F.* × *ananassa* cv. Chandler and *F. vesca* 815 and 112 primer pairs rendered strong amplification products. The remaining 38 primer pairs that either failed to amplify or produced a faint band were then optimized for PCR amplification using the Taguchi method (Cobb and Clarkson 1994), and selected conditions are specified in supplementary Table 1S. Finally, a total of 138 primer pairs (92%) rendered clear amplification products, with 122 (88.4%) producing amplicons of sizes approximately equal to those expected in silico. Since all but two of the sequences used for primer design were ESTs, the failure of amplification or the amplification of larger products could be explained by the presence of introns. Of the 122 new SSR markers developed, 100 were developed from cultivated strawberry sequences (Table 2) whereas 22 were developed from *F. vesca* (Table 3). Among these genic-SSRs, 36.9% were dinucleotide repeats, 55.7% were trinucleotide repeats, 4.9% contained both di- and trinucleotide repeats and one and two were tetra- and pentanucleotide repeats, respectively. For the EST sequences, homology to genes with known function could be established for 81 sequences (67.5%), with 12 markers (10%) in unknown proteins and 27 sequences (22.5%) with no significant similarity found (Tables 2 and 3).

Polymorphism in *F.* × *ananassa* was evaluated in 6–10 cultivars selected because of (1) being a source of ESTs, (2) their diverse origin or (3) being parental lines of various strawberry mapping populations developed in our research centre (Table 1). Only 9 out of the 122 SSR markers (7.4%) did not show polymorphism in the analyzed genotypes. Interestingly, in all cases the SSR motif was a trinucleotide repeated 5–6 times localized in the open reading frame (ORF; Table 2). It has to be noted, however, that 23 other SSR markers were polymorphic even though the SSR motif (trinucleotide repeats in all instances) was located in the ORF. The majority of the polymorphic SSR markers generated more than two bands in each cultivar, representing different alleles at a choice of the four genomes of octoploid strawberry. However, 16 SSR markers (14.2%) amplified only one or two bands in each of the analyzed cultivars, suggesting that they may be specific to one genome. To confirm this, a larger representation of cultivars should be analyzed and the inheritance behaviour tested in an octoploid strawberry segregating population.

Based on the number of amplified alleles, trinucleotide motifs were found to be as equally polymorphic as dinucleotide motifs, as the mean number of alleles generated by polymorphic di- or trinucleotide SSRs (6.29 and 5.27, respectively) was not significantly different ($p = 0.06$). As an example, the four most polymorphic SSR markers generated a total of 14–15 alleles in the analyzed cultivars, with two of

Table 2 Characteristics of 100 strawberry genic-SSR markers developed in this study and amplification in *F.* × *ananassa*, diploid *Fragaria* reference population, diploid *Rosa* and *Rubus*

								F. x ananassa				*Fragaria*		*Rosa*		*Rubus*	
Locus	Accession	Motif	Position[a]	Primer sequence (5'- 3')	Ta (°C)	Size[b]	Putative function	Cultivars	Size range	No. alleles[c]	Alleles/cv[d]	FV x FB[e]	Size range	E15/D10[f]	Size range	Rt/Rm[g]	Size range
ChFaM003	GU815784	(TC)14	5´UTR	**F:** CCTCCCCAACTGATTCTTCA **R:** TGCCATGGTTGTTCCTTCAC	58	114	Iron(III)-zinc(II) purple acid phosphatase	+/+	400-500	8	4-6	+/+	400-460	-/-	-	-/-	-
ChFaM009	GU815785	(AG)7	5´UTR	**F:** TCCAAGTTCCAGAGCTCCTT **R:** CGTCGAGCTCAGACATGAAA	58	181	Translation initiation factor	+/+	520-600	6	2-5	+/+	605-610	-/-	-	-/-	-
ChFaM010	GU815786	(CT)7	5´UTR	**F:** TATCGCCTGCAATTCATCTG **R:** GCTGGCTCTGTGGAGTGAGT	58	162	Unknown protein	+/+	294-310	3	2-3	+/+	312-315	-/-	-	-/-	-
ChFaM011	GU815787	(TC)15	5´UTR	**F:** TCCTCTCCTTCTTTCCCTTCA **R:** CGAGATCTCCCGAGACTGAG	61‡	131	N-acetylglucosaminyl transferase	+/+	166-180	2	1-2	+/-	183	-/-	-	+/+	180-188
ChFaM017	GU815788	(CT)8...(TCG)5	N.P.	**F:** CTCACTCTCTGCGAACTTGC **R:** CAACTCACCTTGCACCGATT	58	128	N.S.	+/+	128-162	3	1-3	+/+	100-172	-/-	-	-/-	-
ChFaM018	GU815789	(TC)8...(TC)5	N.P.	**F:** AGCCGCATCCCTCTTTTCTA **R:** CTAGGGATTGAGGACCGACA	62	217	N.S.	+/+	198-229	8	6-7	+/-	210	-/-	-	+/+	207-217
ChFaM022	GU815790	(AG)24	5´UTR	**F:** GGGCCACTCCTACTTCTTCA **R:** TTGGCCTTGAGAGCTTCGAT	61	212	L-Asparaginase	+/+	198-260	3	2-3	-/-	-	-/-	-	-/-	-
ChFaM026	GU815791	(AAG)14	5´UTR	**F:** TCGTCTCTCGTTTCTCTAAAAGC **R:** ATCAAGTGCTCGTCCACGTA	58	113	Profilin	+/+	92-113	5	3-5	+/+	98-101	-/-	-	-/-	-
ChFaM030	GU815792	(TCTCT)5	5´UTR	**F:** CCATGAAGCAGTGAAGTCCA **R:** AGAAAATCCCGAGAGCCTTT	58	176	Thioredoxin H	+/+	500-510	2	1-2	-/-	-	-/-	-	-/-	-
ChFaM031	GU815793	(CCA)8	ORF	**F:** GCTAGCAAAGCCCTAAGCAA **R:** ACGGTGGGCACACTTAAAGA	58‡	190	Avr9/Cf-9 elicited protein 146	+/+	180-185	4	2-4	+/-	182	-/-	-	-/-	-
ChFaM032	GU815794	(TTC)9	N.P.	**F:** GGTCCCTGCTTCTTCTTCTT **R:** TTCAGCCCCATTTTCCAGTA	59	210	N.S.	+/+	197-220	5	2-3	+/-	195	-/-	-	-/-	-
ChFaM033	GU815795	(CTT)8	5´UTR	**F:** CACAAATCCACACACAGCAG **R:** CCCAGGAAGGTAACCACAAA	60	202	Hydrophobic protein LTI6A	+/+	325-350	3	2-3	+/+	325-350	-/-	-	-/-	
ChFaM035	GU815796	(TCT)9...(TC)8	N.P.	**F:** AACCCACTTCCACAGGTGAC **R:** AGGAAAAAGAGGGCTTGGAG	62	243	N.S.	+/+	239-243	4	1-2	-/-	-	-/-	-	-/-	-
ChFaM036	GU815797	(ATG)9	3´UTR	**F:** GCAGCCTCAAGAAGTGAAGG **R:** CCATCTTGATATCACAGGCATAA	57‡	209	C2/H2 Zn finger transc. factor	+/+	205-215	4	1-2	+/+	177-190	+/+	230-255	-/-	-
ChFaM037	GU815798	(AAG)6	N.P.	**F:** ACGACGATCACCACAAGGAC **R:** AAGCTCCGTCGTTTCCTCTT	62	196	N.S.	+/+	196-204	5	1-4	+/-	195-245	-/-	-	-/-	-
ChFaM039	GU815799	(CTT)7	ORF	**F:** GTGGTTTTTGTTGGGCAAAG **R:** AACGGCTTATCATCCTGCAT	60	184	Mitochondrial carrier protein	+/+	575-600	3	2-3	+/-	575	-/-	-	-/-	-
ChFaM040	GU815800	(TCC)7	ORF	**F:** AGTGGTCATCAGCACCATCA **R:** TAACCGGGAACGGTACTCTG	62	194	60S ribosomal protein L18a	+/+	180-210	6	2-4	+/-	200	-/-	-	-/-	-
ChFaM041	GU815801	(GAG)6	ORF	**F:** CCACACGAAGGAGAAGGAGA **R:** AACGTGTCATTCGACCACAA	60	186	RNA or nucleic acid binding protein	+/+	165-190	8	2-6	+/-	185	-/-	-	-/-	-
ChFaM044	GU815802	(CT)10...(CCG)5	5´UTR	**F:** CGCTGAGTCGTTCTAATTTCA **R:** TTTTGTTGACGAGCGAGATG	58‡	188	Dynamin-like protein	+/+	177-225	5	1-4	+/+	245-280	-/-	-	-/-	-
ChFaM046	GU815803	(CCT)8	ORF	**F:** CCATTTCCATGGCCTTGTTT **R:** GGCCTTGTTGGGTCTGAGAG	61	134	Lil3 protein	+/+	134-154	5	3-5	+/-	100-135	-/-	-	+/-	117
ChFaM047	GU815804	(TC)9	N.P.	**F:** TCATTTCTCTCTCCCCTCGAT **R:** GATGTGATTTACGGCGAAGG	60	207	N.S.	+/+	212-220	7	3-4	-/-	-	-/-	-	-/-	-
ChFaM056	GU815805	(GCA)6	ORF	**F:** AAAACGTCGTCGTTCAGGAT **R:** CGTACTGCTGTTGCTGCTGT	58	208	Nuclear acid binding protein	+/+	202-211	3	1-2	+/-	205	+/-	290	+/+	190-195
ChFaM058	GU815806	(ACC)6	ORF	**F:** GACCCAAACACACCCTGAAC **R:** CTCCCCATTCTGGGAAATCT	59‡	162	Growth-Regulating transcription factor	+/+	162-172	3	2-3	+/-	160	+/-	152	+/-	145
ChFaM060	GU815807	(CCG)6	N.P.	**F:** TGAGCTACCACCAAGAACCC **R:** AATACCCTTGGTACCCCTCG	58‡	201	N.S.	+/+	197-209	4	2-3	+/-	193	-/-	-	-/-	-
ChFaM061	GU815808	(CTT)6	N.P.	**F:** GTGCTCAAGAAACCCTTTCG **R:** GCGCTAGCAACAGTAAGGTG	58	203	N.S.	+/+	190-220	8	2-5	+/+	190	-/-	-	+/+	159-161
ChFaM062	GU815809	(TGA)5...(TTG)6	3´UTR	**F:** GGTTTCGCTTGGAGGATATG **R:** CACCAAATCTCTGATCACAAAAA	58	167	Zinc-finger protein	+/+	155162	4	3-4	+/+	160-170	-/-	-	-/-	-
ChFaM063	GU815810	(CCA)6	ORF	**F:** GACGTCTCCGATCCGTTGAT	61	156	Cysteine proteinase RD19A	+/-	148-158	2	2	+/-	158	+/+	160-166	-/-	-

Table 2 continued

Locus	Accession	Motif	Position[a]	Primer sequence (5'- 3')	Ta (°C)	Size[b]	Putative function	*F. x ananassa*				*Fragaria*		*Rosa*		*Rubus*	
								Cultivars	Size range	No. alleles[c]	Alleles/cv[d]	FV x FB[e]	Size range	E15/D10[f]	Size range	Rt/Rm[g]	Size range
				R: CTGGCTCGCGTACGACTTTC													
ChFaM064	GU815811	(CGA)6	ORF	**F:** CACCAACTTCGACAACCTCA	58	177	Unknown protein	+/+	163-182	5	3-5	+/-	180	+/+	185-192	+/-	183
				R: GACTCTTCTTTGGCGAGCTG													
ChFaM065	GU815812	(GTG)6	ORF	**F:** GACCGGGAGAGATAACAGCA	58	162	Unknown protein	+/+	153-168	6	3-6	+/+	160-165	-/-	-	-/-	-
				R: ATAGAAGCCAATGCGTGATG													
ChFaM066	GU815813	(CTT)5...(GT)8	5´UTR	**F:** ATTTTGGCCACGAAGAATTG	58	232	Putative ERD4 protein	+/+	212-240	7	3-4	+/-	225	-/-	-	-/-	-
				R: CGATGTCAGGAACGAACTGA													
ChFaM067	GU815814	(AAC)5	N.P.	**F:** AGAACCAGCAAGAGCAGCAC	58	171	N.S.	+/+	168-179	4	3-4	+/-	170	-/-	-	-/-	-
				R: CAGCTCTGTGTATGCCTGGA													
ChFaM068	GU815815	(GAA)5	ORF	**F:** CATCTCCAGTTCCTTGGCTC	58	155	serine/threonine-protein kinase	+/+	152-160	3	1-3	+/-	154	+/+	220-232	-/-	-
				R: ACGACCATTCCTCTTCCTCA													
ChFaM069	GU815816	(CCA)5	ORF	**F:** AGACCCTTTGGGAGCTTGAT	59‡	146	RNA-binding protein	+/-	264	1	1	-/-	-	-/-	-	+/-	217
				R: TTCGCAGGCAATTCCTAACT													
ChFaM070	GU815817	(CTG)5	ORF	**F:** AGCATTGAATTCCCAGTTTTG	56‡	191	Staygreen protein	+/-	191	1	1	-/-	-	-/-	-	+/+	193-196
				R: CCTTGCAGCAGGAACTAAGG													
ChFaM072*	GU815818	(CAT)5	N.P.	**F:** TGGCAGAAATTTCCAAAAGG	58	173	N.S.	+/+	350-420	4	3-4	+/+	350-370	+/+	null-340	+/+	355-360
				R: CTCCCCCAGAAGTCCAGATT													
ChFaM074	GU815819	(TGG)5	ORF	**F:** GGTGAGGATGGACTGAAGGA	58‡	159	DnaJ protein homolog ATJ2	+/-	250	1	1	+/-	248	-/-	-	-/-	-
				R: TGAACCAATCCTTTCCAGGT													
ChFaM076	GU815820	(TCG)5	ORF	**F:** GCCTCCATGGAAAACCTAAA	58	143	TINY transcription factor	+/+	146-152	3	1-2	+/+	115-148	-/-	-	-/-	-
				R: CTCGGCAGCTCCACTATCTC													
ChFaM077	GU815821	(ATG)5	3´UTR	**F:** GAAAGGGCTGGACATGGATA	57	225	Plasma Membrane H+ ATPase	+/+	216-228	4	3-4	+/-	228	-/-	-	-/-	-
				R: ATGTTGTTATTTGGCCTGCT													
ChFaM078	GU815822	(CCT)5	N.P.	**F:** CAGCCTCATTGCAAATCTGA	60	250	N.S.	+/+	249-255	2	1-2	+/+	255-260	-/-	-	-/-	-
				R: CTTACCGGTTTCGATGTGGT													
ChFaM079	GU815823	(ACT)5	3´UTR	**F:** TCGAGTTCTACGCTTGCTGA	60	192	SAUR family Auxin-induced protein	+/+	195-210	6	2-5	+/-	200	+/-	202	+/-	217
				R: CCGTGCAATCAAATCACACT													
ChFaM080	GU815824	(GAC)5	3´UTR	**F:** TTCGGTGCCGGTAAAGATAC	60	219	Unknown protein	+/+	198-222	4	2-3	+/-	230	-/-	-	-/-	-
				R: AAGTTCCACCACCATGCAAT													
ChFaM081	GU815825	(GTC)5	ORF	**F:** AACTGAGCTCTCGGCAAGTC	56	191	Prenylcystein oxidase	+/-	191	1	1	+/-	198	-/-	-	-/-	-
				R: GAATACTCGCGGAGGAAGTG													
ChFaM082	GU815826	(GAG)5	ORF	**F:** AGTACGGCAATTGAAGCTCG	60	181	Unknown protein	+/-	181	1	1	-/-	-	-/-	-	-/-	-
				R: GATCTCGGTTGAAGATCGGA													
ChFaM083*	GU815827	(ACT)5	5´UTR	**F:** TTTTCTGGCTTTTGGATTGG	57	191	Zinc binding transcription factor	+/+	191-193	2	1-2	+/+	194-198	+/-	195	+/-	188
				R: GCACTTTTTCATCACAGGCA													
ChFaM085	GU815828	(GGT)5	ORF	**F:** AGATGGGTCATTTTCTGACGA	61	164	Purple acid phosphatase	+/+	151-174	5	2-4	+/-	167	-/-	-	+/-	148-169
				R: GTAGTGCATGTCCGCCACTT													
ChFaM086	CO816743	(GGT)5	ORF	**F:** TTTGGAGCTCAATCCCATCTG	61	208	N.S.	+/+	204-214	3	2-3	+/-	215	-/-	-	-/-	-
				R: ATTTGGCCAGCCTCCGTCT													
ChFaM088	GU815829	(GCC)5	ORF	**F:** GGTGGCAAAACTCATGGAGA	57‡	164	Unknown protein	+/+	154-178	5	3-5	+/-	168	-/-	-	-/-	-
				R: GGGAAGCGAAGTTGAAGAGG													
ChFaM089	GU815830	(CGA)5	ORF	**F:** GAAGGATGGTTGCACACAGG	60	152	4-coumarate-CoA ligase	+/-	152	1	1	+/-	154	+/-	155	+/-	154
				R: GAGAGGTTGGGATGGGAGAT													
ChFaM091	GU815831	(AGA)5	ORF	**F:** CATTGTTTGCTGCTCCCAAG	59‡	178	Zinc finger protein	+/-	178	1	1	+/-	178	-/-	-	-/-	-
				R: TTCCTAATTGCTCCCTGCAA													
ChFaM092	GU815832	(TGC)5	N.P.	**F:** ACCCAAGTTCCCTTCGACTC	60	130	N.S.	+/+	110-130	6	1-6	+/+	130-135	-/-	-	-/-	-
				R: ATGCGCTTTGCATAACAGGT													
ChFaM093	GU815833	(CTC)5	N.P.	**F:** CGCCCTCAAATCCCTCTAAC	60	214	N.S.	+/+	298-304	2	1-2	+/-	330	-/-	-	-/-	-
				R: GAAGTGAGTGTTCCGCTGCT													
ChFaM094	GU815834	(GAA)5	ORF	**F:** ATGGAGGGCGCTACTGAAAA	61	126	tRNA isopentenylpyrophosphatase	+/+	126-134	5	3-4	+/-	130	-/-	-	-/-	-
				R: AATGGCGAGCTTGGACTTTC													
ChFaM095	GU815835	(AG)7	5´UTR	**F:** GCCAGAAGCAAAAACCAGAA	58	164	Signal peptidase protein	+/+	138-170	7	4-6	+/+	168-170	-/-	-	-/-	-
				R: GGGAAGTTGAAATTGTCGGA													
ChFaM097	GU815836	(TC)7	5´UTR	**F:** GCCAAAGGTTGGTTCTTTGA	58	236	Unknown protein	+/+	236-260	6	1-3	+/-	280	-/-	-	-/-	-
				R: GCCATTTTGGAAGAGGTGAA													
ChFaM098	GU815837	(TC)7	5´UTR	**F:** GTGAGAGTCAGCCCACCCTA	58	214	Unknown protein	+/+	190-270	14	3-6	+/+	218-220	+/+	350-400	-/-	-

Table 2 continued

Locus	Accession	Motif	Position[a]	Primer sequence (5'- 3')	Ta (ºC)	Size[b]	Putative function	*F. x ananassa*				*Fragaria*		*Rosa*		*Rubus*	
								Cultivars	Size range	No. alleles[c]	Alleles/cv[d]	FV x FB[e]	Size range	E15/D10[f]	Size range	Rt/Rm[g]	Size range
				R: GCGACGAGGATGAAGAAGAG													
ChFaM100	GU815838	(CT)7	N.P.	**F:** TTGGAACCCAGAAAATCGAA	60	121	N.S.	+/+	111-130	9	5-8	+/-	123	-/-	-	-/-	-
				R: CAGCGGAGAGAGAAACAAGG													
ChFaM101	GU815839	(AG)7	5´UTR	**F:** GGAGTAAGCTGATCACTCTGT	58	157	3-Methylcrotonyl-CoA Carboxilase	+/+	150-180	8	3-6	+/+	158-168	+/+	152-182	-/-	-
				R: ACTCCGAGGCTGTAATCCCT													
ChFaM103	GU815840	(CT)7	5´UTR	**F:** CATCTCTTCTCCTTTCCGATCT	58	160	Delta-7-sterol-C5(6)-desaturase	+/+	122-178	4	2-4	+/+	152-162	-/-	-	-/-	-
				R: GAGCACAATGCGGTTGTAGA													
ChFaM104	GU815841	(TA)7atct(TA)10	N.P.	**F:** CAGTCATTTTTGGCTTCACC	59‡	203	N.S.	+/+	175-210	10	2-6	+/-	190	-/-	-	-/-	-
				R: TTGGTCTGTTCCTTTCCTTG													
ChFaM105	GU815842	(CT)7	5´UTR	**F:** CCTCCAAACACAATCCACCA	57‡	198	MYB transcription factor	+/+	198-212	7	2-4	+/-	215	-/-	-	-/-	-
				R: TCTGAGGTTTATGCGGGACT													
ChFaM106	GU815843	(AG)7	5´UTR	**F:** ACCAACCGAGGCGAGAGAG	59	139	Serine/threonine protein phosphatase	+/+	125-158	8	4-8	+/+	138-142	-/-	-	-/-	-
				R: CGTCATCTGCACCTGCTTC													
ChFaM107	GU815844	(AT)7	N.P.	**F:** TGCCAAACAAACAAATGTTGA	58	196	N.S.	+/+	175-205	5	2-3	+/+	195-200	-/-	-	-/-	-
				R: CATATCGATGTCCTTCATAGGG													
ChFaM108	GU815845	(GA)6	N.P.	**F:** CATGGAAAGAGAGCGAAAGC	60	171	RabGAP/TBC domain-containing protein	+/+	170-185	6	2-6	+/-	175	-/-	-	-/-	-
				R: CGAGGAATGAGGGAGGAATC													
ChFaM109	GU815846	(TA)6	3´UTR	**F:** AAGCTGCTTGCGAAGAACAA	61	150	TA4 transcriptional activator	+/+	145-180	5	3-5	+/-	155	+/-	140	-/-	-
				R: GGCTGATGCCAGTCCATTTA													
ChFaM110	GU815847	(TC)6	N.P.	**F:** TTTTCCTTTGGTGGTTTTGG	57‡	161	N.S.	+/+	null-168	2	0-1	+/-	165	-/-	-	+/-	190
				R: CCAGAGAAAGCCATTTAGGC													
ChFaM111	GU815848	(TC)8...(TC)6	N.P.	**F:** GCCCAACCGAGTCTCTCTCT	61	188	N.S.	+/+	188-210	7	2-4	+/+	350	-/-	-	-/-	-
				R: CGGGCTTCAATTTGCTCAAT													
ChFaM112	GU815849	(TC)6	N.P.	**F:** TTCAAGCTTTTCTTCTTCCTCTC	62	182	N.S.	+/+	180-190	7	4-5	+/+	182-188	-/-	-	-/-	-
				R: CATCTTCACCTGACCCCTCT													
ChFaM114	GU815850	(TA)6	3´UTR	**F:** TGTGGTGCCTTGGTTTACAA	62	209	Membrane intrinsic protein	+/+	198-248	3	1-3	+/+	210-230	+/+	null-172	-/-	-
				R: CGCCTCTCTCCTCTCTAATCC													
ChFaM115*	GU815851	(GA)6	3´UTR	**F:** CCTCGGCTTCTTCATCTTTG	61	136	Unknown protein	+/+	350-650	3	2-3	+/+	620-650	+/+	350-450	+/-	142
				R: ACCACACTGACAGGACGATG													
ChFaM117	GU815852	(AAC)5	ORF	**F:** CCAAGGCGATTCTATCTTGC	58	156	Legumin-like protein (prunin)	+/+	162-202	3	2-3	+/-	144-200	-/-	-	+/-	186
				R: GAGGAGTCTGGTGTCGAAGC													
ChFaM120*	GU815853	(TTC)5	5´UTR	**F:** GGTTTCATCAGAGGGCGTCTT	59‡	162	Tubulin alpha-2 chain	+/+	163-194	15	4-9	+/+	180-185	+/+	175-185	+/+	180-190
				R: TAAAGCTCCCAGCAGGCATT													
ChFaM122	GU815854	(AAG)5	5´UTR	**F:** GACTCACAGTCTCTCCCAGTGT	56‡	140	Amino acid selective channel protein	+/+	135-165	5	2-3	+/-	144	-/-	-	+/-	700
				R: TGATATTGAGCAACGGGTGA													
ChFaM126	GU911465	(AGG)6	ORF	**F:** GTTAGGGAGTCCGGGAATGA	61	191	Abscisic acid responsive bZIP protein	+/+	185-230	5	3-5	+/+	190-193	-/-	-	-/-	-
				R: TTCCAATCCCATCTGACGAC													
ChFaM129	GU815855	(TC)6	N.P.	**F:** AGATCAACATCGCCTCCAAC	60	190	N.S.	+/+	194-230	7	1-6	+/+	205-210	-/-	-	-/-	-
				R: TGCTCGTTGTCCATAACCTG													
ChFaM130	GU815856	(CCT)6	ORF	**F:** GCCAGTCACAAGAAGCCAAA	61	202	C2/H2 Zn finger transcription Factor	+/+	199-211	5	3-5	+/+	215-225	+/-	190	-/-	-
				R: TGCTCTGAAACCCCTTTATCTG													
ChFaM138	GU815857	(CCG)6	5´UTR	**F:** GGCAGTAACCTCCAGAACTCTAA	61	214	10 kDa chaperonin	+/+	298-300	2	1-2	-/-	-	-/-	-	-/-	-
				R: TCGCATTTCCAGCCTTATCT													
ChFaM141	GU815858	(GA)7	5´UTR	**F:** AAAAGAAGGGAAAAGAAATTGG	57‡	142	Monocopper oxidase-like protein	+/+	137-144	4	3-4	+/-	147	+/+	140-153	-/-	-
				R: ACTCAAGCTCGCAAAGAAGC													
ChFaM142*	GU815859	(TTC)5	5´UTR	**F:** TGCTCAGCTGCTTCTTGCTC	59	188	Terminal flower 1 like	+/+	182-209	4	3-4	+/+	200-210	+/-	180	+/-	177
				R: CAGCCATTGGTGACATGCTT													
ChFaM144	GU815860	(CAC)5	ORF	**F:** CACGTCTTGGCTTCCTCTTC	58	211	Isopropyl malate dehydratase	+/-	282	1	1	+/-	290	-/-	-	-/-	-
				R: CGGAGCAGATTTTCTTGGAG													
ChFaM145	GU815861	(CAC)6	N.P.	**F:** CCACCGAAGAACCAAAAGAA	58	128	N.S.	+/+	115-180	6	3-5	+/+	128-140	+/-	107	-/-	-
				R: AACTGGTGCAACTGGAGCTT													
ChFaM146	GU815862	(GGA)5	ORF	**F:** ACGAGGGAAGAATGGAGCAC	58	124	Unknown protein	+/+	120-128	3	2-3	+/-	117-125	+/+	null-162	-/-	-
				R: AGATGGTCCTGACTGGATGG													
ChFaM147	GU815863	(AG)8	5´UTR	**F:** ACGAGGGTCACCTGAGACTG	58	222	NADH dehydrogenase	+/+	218-222	3	1-3	+/+	220-225	-/-	-	-/-	-
				R: CCAGGAGAAGGTACCGAAGG													
ChFaM148	GU815864	(AT)7	Intron 1	**F:** CCCTCCATCAAAGCCAGTT	58	158	GALUR	+/+	156-188	9	3-6	-/+	150	-/-	-	-/-	-

Table 2 continued

Locus	Accession	Motif	Position[a]	Primer sequence (5'- 3')	Ta (ºC)	Size[b]	Putative function	*F. x ananassa*				*Fragaria*		*Rosa*		*Rubus*	
								Cultivars	Size range	No. alleles[c]	Alleles/cv[d]	FV x FB[e]	Size range	E15/D10[f]	Size range	Rt/Rm[g]	Size range
				R: CATTAGACCCCGACTTGTCA													
ChFaM151	GU815865	(CT)7	5´UTR	**F:** ACCACCACCGTTTTCTCCTC	60	211	Unknown protein	+/+	208-220	4	2-3	+/+	212-224	+/+	250-275	-/-	-
				R: ACCACCGACTCGTCCTTCTT														
ChFaM159	CO817345	(CT)13	5´UTR	**F:** TCTCTCTCATCGCCCCAGAG	63	136	Ubiquitin-conjugating enzyme E2	+/+	251-262	6	3-5	+/+	259-261	+/+	248-255	-/-	-
				R: ACCCATCCACAGGGTTCTTG														
ChFaM160	GU815866	(ATG)7	ORF	**F:** CCACTCCCCAAAAGAGCACA	62	190	ABC-type Co2+ transport protein	+/+	178-193	5	3-5	+/+	188-191	+/-	180	-/-	-
				R: CTGCCTCCACAATCCTCACC														
ChFaM161	CO816938	(TTC)7	5´UTR	**F:** CGAGGCCTTGTCTTCTTTGT	61	206	NAD-dependent glucuronic acid epimerase	+/+	188-219	8	4-6	+/+	208-230	-/-	-	-/-	-
				R: GCGGAGGTAGCTGTTGTAGC														
ChFaM163	CO381605	(AT)9	N.P.	**F:** TTCGGGTCTTGTATTGCTTTGA	59	118	N.S.	+/+	109-129	6	3-4	+/-	112	-/-	-	-/-	-
				R: TTCAATTCCGAAGGCACAAC														
ChFaM164	GU815867	(CT)8	5´UTR	**F:** CACTCAGCCAGATCCAGAGC	63	151	60s acidic Ribosomal protein	+/+	153-300	14	8-10	+/+	178-248	-/-	-	-/-	-
				R: GCGCCAAGGATGGTCTTAAT														
ChFaM170	GU815868	(GTGA)4	3´UTR	**F:** CGGGAGAAAATGAAAAAGGA	57	168	SCARECRAW transcription factor	+/+	170-172	2	1-2	-/-	-	-/-	-	-/-	-
				R: TACTTGGTTCCGGCAACACT														
ChFaM174	GU815869	(CT)17	N.P.	**F:** GAGGGAGATGGCACGGAGAT	61	156	N.S.	+/+	152-220	5	2-3	+/+	185-260	+/+	188-208	-/-	-
				R: CTCCGGCATTGAAATCGAGA														
ChFaM177	GU815870	(AAG)5	3´UTR	**F:** CCTGCAGAGTGCAAGAGAGA	63	143	Xyloglucan endotransglucosylase hydrolase	+/+	140-170	4	3-4	+/+	146-162	-/-	-	-/-	-
				R: CACTCACAAGGCATGAGAGCTA														
ChFaM178	GU911466	(GA)16	5´UTR	**F:** AAACGAAGAAAGGAAGTCTTCAA	61‡	136	Invertase/pectinesterase inhibitor	+/+	114-146	5	2-3	+/-	115	+/+	120-135	+/-	237
				R: AGCTGTGCAGGGGTTTATGT														
ChFaM194	CO379682	(CT)15	5´UTR	**F:** CCCTTCCTTGCCAGTCATTT	61	193	Cinnamyl alcohol dehydrogenase	+/+	190-215	8	3-8	+/-	213	+/-	202	-/-	-
				R: CCCATCCCACAGTTGTTCTC														
ChFaM195	GU911467	(GA)8	3´UTR	**F:** CAAGCTGAAGAAGGGTTGTG	58	210	Phosphoenolpyruvate carboxylase kinase	+/+	205-215	2	1-2	+/+	210-220	-/-	-	-/-	-
				R: GCATTGGTTCTCTTTTCAGGA														
ChFaM196	GU911468	(CTT)14	5´UTR	**F:** CCTCTCACCTCTTTCTCTCTAAAA	60	156	RNA-binding protein	+/+	118-158	9	4-6	+/+	150-155	-/-	-	-/-	-
				R: AGCGCATGAGTCTGCTGTTA														
ChFaM203	CO380455	(GA)17	N.P.	**F:** CGAGGGTCACGGCTACTAAA	58	169	N.S.	+/+	150-210	5	3-4	+/-	150	+/+	160-174	+/+	162-168
				R: TGAGTATGACCAATCCGAAAGA														
ChFaM208*	GU815871	(CTT)5	5´UTR	**F:** TTTCTCTCTTCTCTCTCTCTCACTG	56	228	SGT1 related protein	+/+	232-240	4	1-2	+/+	232-235	+/+	185-285	+/-	260
				R: TCGGTGAGATTGTTGGATTT														
ChFaM209	GU815872	(CTCTT)4	5´UTR	**F:** CCCCCAAAACCCTCTATTCT	60	144	Homeobox transcription factor	+/+	135-155	4	3-4	+/+	124-142	+/+	145-160	-/-	-
				R: ATCATTCCCAAGCCACTGTC														
ChFaM214	GU815873	(AGA)5	5´UTR	**F:** CTCACCACAACTGCAACACC	59	169	Membrane channel protein	+/+	153-186	6	2-4	+/-	162	-/-	-	-/-	-
				R: TGATATTGAGCAACGGGTGA														

*Transferable markers across the *Rosoideae* genera mapped in *Fragaria*.
‡Markers with optimized PCR conditions (Table 1S).
[a]Location of the SSR with respect to the gene. When it has not been possible to determine, N.P. is indicated.
[b]The expected PCR product size in base pairs based on the sequence from which each primer pair is derived.
[c]Total number of bands identified in the total number of analyzed cultivars.
[d]Number of bands identified in each cultivar.
[e]Amplification and polymorphism in the parental lines of the diploid *Fragaria* reference segregating population. When a primer pair amplified a product in any genotype, a '+' sign is indicated. If a primer pair also detected polymorphisms a '+/+' sign is indicated. When primer pairs did not amplify in *F. vesca* but amplified a product in *F. bucharica*, a '-/+' sign is indicated.
[f]Amplification and polymorphism in *Rosa wichurana* (E15) and 'Blush Noisette' (D10).
[g]Amplification and polymorphism in *Rubus idaeus* cultivars 'Tulameen' (Rt) and 'Miraz' (Rm).

Table 3 Characteristics of 22 *F. vesca* SSR markers developed in this investigation and amplification in *F.* × *ananassa*, diploid *Fragaria* reference population, diploid *Rosa* and *Rubus*

								F. x ananassa				*Fragaria*		*Rosa*		*Rubus*	
Locus	Accession	Motif	Position[a]	Primer sequence (5'- 3')	Ta (ºC)	Size[b]	Putative function	Cultivars	Size range	No. alleles[c]	Alleles/cv[d]	FV x FB[e]	Size range	E15/D10[f]	Size range	Rt/Rm[g]	Size range
ChFvM028	DV438195	(CTT)6	N.P.	F: AATGGCATCAACTTCTGCAC R: CAGCCTGCTGCTGTAGTTCT	58	175	Unknown protein	+/+	160-180	5	4-5	+/+	173-176	+/+	175-185	-/-	-
ChFvM049	EX671238	(AAG)7	3´UTR	F: ATGGTTGGTGATCAATGGTG R: TTCATATGCAATTTGATGGAAC	58	159	Metal ion binding protein	+/+	151-173	7	3-4	+/+	156-160	-/-	-	-/-	-
ChFvM050	DY674195	(TC)22	5´UTR	F: CCCTTCCCTTTCCTTTCCTT R: TAATGGGGTGGTGGAGTTCA	57‡	156	Fe-S metabolism associated protein	+/+	120-156	11	5-8	+/+	140-150	-/-	-	-/-	-
ChFvM075	DY675833	(AAC)9	N.P.	F: AGGAAGAGGACGAGGACGAA R: ACCTGCATTCCCTCCTGATT	61	226	R3H domain protein	+/+	420-470	2	1-2	+/+	460-470	-/-	-	-/-	-
ChFvM084	DY675569	(TTGA)5(GA)6	5´UTR	F: CCAAACAGTCCTCACGGTTC R: GTAGCACCAGCAACATGACG	61	151	RING-H2 finger protein	+/+	150-158	4	2-4	+/-	154	-/-	-	-/-	-
ChFvM087	DY675366	(GAA)9	5´UTR	F: GAAAGGGGAAGCCTTTTCAT R: TGGGACGAAAGTTCCCAATA	60	224	Acid phosphatase	+/+	208-239	6	3-5	+/+	212-232	-/-	-	-/-	-
ChFvM125	EU024861	(AG)9	fosmid	F: GCGACTGCCATCGTAACCTA R: TCTCCAATGTTTTCGCCAAG	60	167	10 Kb of CONSTANS	+/+	160-210	4	2	-/+	175	-/-	-	-/-	-
ChFvM140	DY674556	(TC)16	5´UTR	F: CCACTTCCCATACCACTGGA R: CGTCGTAGGTTCGGTCTCTG	61	131	Sucrose cleavage protein	+/+	94-137	12	2-10	+/+	122-137	+/+	137-187	-/-	-
ChFvM181	DY668343	(GGT)5cgc(GGT)6	N.P.	F: GGAGAACTGCTTTGGTAGGG R: ACGTACACCAGCAGATGAGC	59‡	153	N.S.	+/+	124-178	7	3-5	+/+	160-170	-/-	-	-/-	-
ChFvM182	DY673363	(CAA)6...(ACA)5	5´UTR	F: GGAACCAAACGAACACCAAC R: GCGGAGGAGTGAGTGAAGAC	63	210	AT-Hook DNA-Binding Protein	+/+	190-240	7	4-6	+/+	230-236	-/-	-	-/-	-
ChFvM184	DY667864	(GAT)12ggtgg(TGA)6	ORF	F: GCACTTTGGGGTTTTGGGTA R: TTGCAAGAACCCCCTTCCTA	61	244	F-box family protein	+/+	198-248	9	3-5	+/+	228-240	-/-	-	-/-	-
ChFvM191	DY673483	(CAA)7(CAG)15	ORF	F: CCAGCAGAATCCTCAAATGC R: GTGCCAACAAGCCCATTG	61	231	WD-40 repeat protein (*STYLOSA*)	+/+	205-255	8	3-4	+/+	200-240	+/+	210-235	-/-	-
ChFvM192	DY668080	(ACA)5...(CCA)5	ORF	F: TGAGGTCAGTCTGTGAGAATTG R: TTTCCGAGAGTGGAGAGCAT	57‡	200	bHLH transcription factor	+/+	198-285	14	6-10	+/-	208	-/-	-	+/+	700-800
ChFvM193	DY669902	(ATC)5(TC)21	N.P.	F: CATCAGAACCATCAATCATCG R: TACTGCCGGAGAATGAACA	56‡	178	N.S.	+/+	166-182	6	3-6	+/+	175-185	+/+	185-218	-/-	-
ChFvM201	CX661912	(TC)10	5´UTR	F: TGATTCAACTCCAGCGAAGC R: ATCAGCAGGCGAATCCTTCT	61	161	Ankyrin domain protein	+/+	220-280	9	4-7	+/+	260-270	-/-	-	-/-	-
ChFvM202	DY673477	(GA)21	5´UTR	F: CAAAGGGCTCCAGCTATCTC R: AGGATCGTCAGCTAAGAAGCA	62	185	Fatty acid hydroxylase	+/+	150-190	6	3-5	+/+	195-200	-/-	-	-/-	-
ChFvM205	DY672089	(ATT)14	3´UTR	F: GCGAAACCCTATGGATTGTT R: ACAACCACCAAATTCCCATT	61	152	Peptidyl-prolyl cis/trans isomerase	+/+	112-172	6	3-4	+/+	130-153	-/-	-	-/-	-
ChFvM206	DY675467	(CT)19	5´UTR	F: CCGAGAGAGCCATAACCACT R: GTTTATAGGCGTGGGCTCTG	57‡	154	Nucleic acid binding protein	+/+	200-230	6	2-4	+/+	235-250	+/+	218-238	-/-	-
ChFvM207	DY671769	(AGA)12	5´UTR	F: TAGTCGCCCCATTCCTTTCT R: ACAGAGCACCAAACCCACAC	61	133	Ankyrin repeat family protein	+/+	500-650	5	4-5	+/-	550	-/-	-	-/-	-
ChFvM210	DY675018	(TC)23	5´UTR	F: TCCCCACATTTTCCTTGTTT R: GTGGTGTTGGTGAGTGGAGA	59	220	Auxin-regulated protein	+/+	190-220	5	2-5	+/+	180-192	-/-	-	-/-	-
ChFvM212	DY673712	(TCT)7	5´UTR	F: CAAATCTTCAACGGTCTCTCC R: ACGGAGGAGGAGGAAGTCAT	60	115	Transketolase	+/+	95-135	6	3-6	+/+	118-140	-/-	-	-/-	-
ChFvM213*	DY672560	(GA)9	5´UTR	F: AACCCTAGGAGGCTGAAACC R: ATACCCCGGGTGGTACTTGT	60	215	60S Ribosomal protein L27A	+/+	218-250	4	2-3	+/+	220-236	+/-	215	+/-	215

*Transferable markers across the *Rosoideae* genera mapped in *Fragaria*.

‡Markers with optimized PCR conditions (Table 1S).

[a]Location of the SSR with respect to the gene. When it has not been possible to determine, N.P. is indicated.

[b]The expected PCR product size in base pairs based on the sequence from which each primer pair is derived.

[c]Total number of bands identified in the total number of analyzed cultivars.

[d]Number of bands identified in each cultivar.

[e]Amplification and polymorphism in the parental lines of the diploid *Fragaria* reference segregating population. When a primer pair amplified a product in any genotype, a '+' sign is indicated. If a primer pair also detected polymorphisms a '+/+' sign is indicated. When primer pairs did not amplify in *F. vesca* but amplified a product in *F. bucharica*, a '-/+' sign is indicated.

[f]Amplification and polymorphism in *Rosa wichurana* (E15) and 'Blush Noisette' (D10).

[g]Amplification and polymorphism in *Rubus idaeus* cultivars 'Tulameen' (Rt) and 'Miraz' (Rm).

them consisting of dinucleotide and two of trinucleotide repeats. A positive correlation was found when the number of observed alleles was analyzed in relation to the number of repeat units ($r = 0.20$, $p < 0.05$). When the correlation was investigated separately for di- and trinucleotide repeats, a positive correlation was still found for trinucleotides ($r = 0.32$, $p < 0.01$) but not for di-nucleotide repeats ($r = 0.08$, $p = 0.57$).

Transferability between *Fragaria* species and mapping in diploid *Fragaria*

In order to evaluate the polymorphism in the diploid *Fragaria* reference population, the developed primer pairs were used to genotype the *F. vesca* 815 parental line and the F_1 hybrid. Among the 100 *F.* × *ananassa* newly developed SSRs, 90% amplified in *F. vesca* fragments in the same range of size as that in *F.* × *ananassa*. Therefore, ten strawberry primer pairs failed to amplify in *F. vesca* 815, suggesting that either these sequences have diverged between both species or are not present in *F. vesca*. Consistent with the idea that *F. vesca* contributes to the *F.* × *ananassa* genome, all 22 *F. vesca* SSR markers (100%) amplified in *F.* × *ananassa*. Strikingly, the *F. vesca* SSR ChFvM125 did not amplify in *F. vesca* 815, suggesting the presence of a null allele in this accession. We further analyzed the ten strawberry SSR markers failing to amplify in *F. vesca* 815 in four other accessions of *F. vesca* and two accessions of *F. iinumae*. These were *F. vesca* 14 from Huesca, Spain, *F. vesca* 184, *F. vesca* Reine des vallées 660, the white-fruited *F. vesca* 596 and two *F. iinumae* accessions (Table 1). In order to discard technical problems and to be certain about primer specificity, PCR amplifications were tested as least twice for all accessions. Three markers (ChFaM035, ChFaM138 and ChFaM170) did not amplify in any of the *F. vesca* or *F. iinumae* accessions, suggesting that they are specific for *F.* × *ananassa.* Markers ChFaM030, ChFaM148 and ChFv125 amplified some accessions of both *F. vesca* and *F. iinumae*. Markers ChFaM022 and ChFaM069 were specific for *F. vesca*, failing to amplify any of the *F. iinumae* accessions, while markers ChFaM047 and ChFaM70 specifically amplified one or both accessions of *F. iinumae* (Supplementary Table 2S). Therefore, our results indicate that two markers might be specific to the Y1 genomic pool, or derived from an *F. vesca* ancestor, while other two are Z genome specific, or common to *F. iinumae*.

Out of the 122 primer pairs developed here, nine did not amplify in either *F. vesca* 815 or *F. bucharica* 601 (Table 2). Among the remaining markers, 63 primer pairs (56%) revealed polymorphisms and were analyzed in the bin set. In addition, five public but unmapped SSR markers were also mapped in this study (Materials and Methods). A total of 72 loci were mapped in the diploid *Fragaria* reference map. Sixty-three markers amplify one locus and five markers, ChFaM017, ChFaM076, ChFaM140, ChFaM164 and Fvi9, amplify two loci (labelled as a and b). In this case, one (ChFaM076) or both (others) were bin-mapped (Fig. 1). Two markers, ChFaM010 and ChFaM114, did not correspond to identified bins and were mapped in the whole FV × FB mapping population. ChFaM010 defined a new mapping bin in linkage group VII:68 and ChFaM114 defined a new bin in linkage group III:20. To verify the segregation of ChFaM017a, this marker was also scored in the entire population mapping at position IV:26. Also, the dominant marker ChFvM125 was scored in the entire population mapping in position VI:87, cosegregating with marker EMFv010.

The FV × FB bin map is at present composed of 49 bins, spanning 604 cM and with an average of seven bins per linkage group (Fig. 1). The 72 new loci were distributed across the seven linkage groups. Fourteen markers were assigned to four bins on linkage group I, 12 markers to five bins in linkage group II, 12 markers to five bins in linkage group III, 11 markers to three bins in linkage group IV, six markers to four bins in linkage group V, 12 markers to five bins in linkage group VI and four markers to three bins in linkage group VII. The two new mapping bins, defined by markers ChFaM114 and ChFaM010, were localized in linkage groups III and VII, respectively. Both markers fill regions of the *Fragaria* reference map with a reduced number of markers, dividing gaps of 16 cM in two bins of 8 cM for ChFaM114 and 26 cM to 6 and 20 cM for ChFaM010.

Transferability of *Fragaria* SSRs to other Rosoideae species

The genic-SSRs developed in this study together with other 52 published SSRs (Table 4) were

Table 4 Other *Fragaria* SSR markers analyzed for transferability to *Rosa* and *Rubus*

Locus	Reference	*Fragaria*			*Rosa*		*Rubus*	
Genic-–SSRs		*F.* × *a.* cv.[a]	Size range	FV × FB	E15/D10	Size range	Rt/Rm	Size range
ARSFL_31	Lewers et al. (2005)	+/+		+/+	−/−	–	−/−	–
ARSFL_35*	Lewers et al. (2005)	+/+	210–212	+/+	+/+	185–195	+/+	210–212
ARSFL_99	Lewers et al. (2005)	+/+		+/+	−/−	–	−/−	–
ChFaM001	Gil–Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM002	Gil-Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM004	Gil-Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM005	Gil-Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM007	Gil-Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM008	Gil-Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM014	Gil-Ariza et al. (2006)	+/+		±	−/−	–	−/−	–
ChFaM021	Gil-Ariza et al. (2006)	+/+		−/−	−/−	–	−/−	–
ChFaM023	Gil-Ariza et al. (2006)	+/+		+/+	−/−	–	−/−	–
ChFaM029*	Gil-Ariza et al. (2006)	+/+	147–182	+/+	+/+	170–180	±	184
FAC-003a	Lewers et al. (2005)	+/+		+/+	−/−	–	−/−	–
FAC-005b	Lewers et al. (2005)	+/+		+/+	−/−	–	−/−	–
FAC-006a	Lewers et al. (2005)	+/+		−/−	−/−	–	−/−	–
PBCESSRFXA12*	Keniry et al. (2006)	+/+	180–213	+/+	±	172	±	176
UFFa01E03	Bassil et al. (2006a)	+/+		+/+	−/−	–	−/−	–
UFFa01H05	Bassil et al. (2006a)	+/+	250–290	+/+	+/+	230–240	−/−	–
UFFa02H04	Bassil et al. (2006a)	+/+		+/+	−/−	–	−/−	–
UFFa03B05	Bassil et al. (2006a)	+/+		+/+	−/−	–	−/−	–
UFFa15H09	Bassil et al. (2006a)	+/+		+/+	−/−	–	−/−	–
UFFa16H07*	Bassil et al. (2006a)	+/+	235–290	+/+	±	240	+/+	237–244
Genomic-SSRs								
ARSFL_007*	Lewers et al. (2005)	+/+	225–275	+/+	+/+	218–220	±	205
ARSFL_12	Lewers et al. (2005)	+/+		+/+	−/−	–	−/−	–
BFACT-002	Rousseau-Guetin et al. (2008)	+/+	133–253	+/+	+/+	190–202	−/−	–
BFACT-008	Rousseau-Guetin et al. (2008)	+/+		+/+	−/−	–	−/−	–
BFACT-029	Rousseau-Guetin et al.(2008)	+/+	220–240	+/+	−/−	–	+/+	240–270
BFACT-036	Rousseau-Guetin et al. (2008)	+/+	106–178	+/+	+/+	235–260	−/−	–
BFACT-037	Rousseau-Guetin et al. (2008)	+/+		−/−	−/−	–	−/−	–
BFACT-039	Rousseau-Guetin et al. (2008)	+/+		+/+	−/−	–	−/−	–
BFACT-045	Rousseau-Guetin et al. (2008)	+/+		+/+	−/−	–	−/−	–
CFVCT-003	Monfort et al. (2006)	+/+		+/+	−/−	–	−/−	–
CFVCT-005A	Monfort et al. (2006)	+/+		+/+	−/−	–	−/−	–
CFVCT-017	Monfort et al. (2006)	+/+		+/+	−/−	–	−/−	–
CFVCT-027	Monfort et al. (2006)	+/+		+/+	−/−	–	−/−	–
EMFn049*	Sargent et al. (2004)	+/+	176–216	+/+	±	180	+/+	220–282
EMFn160	Sargent et al. (2006)	+/+		+/+	−/−	–	−/−	–
EMFv008	James et al. (2003)	+/+		+/+	−/−	–	−/−	–
EMFv010	James et al. (2003)	±	234	+/+	−/−	–	±	205–275
EMFv023	Hadonou et al. (2004)	+/+		+/+	−/−	–	−/−	–
EMFv027	Hadonou et al. (2004)	±		+/+	−/−	–	−/−	–

Table 4 continued

Locus	Reference	*Fragaria*			*Rosa*		*Rubus*	
Genic-–SSRs		*F.* × *a.* cv.[a]	Size range	FV × FB	E15/D10	Size range	Rt/Rm	Size range
EMFv029	Hadonou et al. (2004)	+/+		+/+	−/−	–	−/−	–
EMFvi018	Sargent et al. (2003)	+/+		+/+	−/−	–	−/−	–
EMFvi108*	Sargent et al. (2003)	+/+	180–210	+/+	±	198	±	190
EMFvi136	Sargent et al. (2003)	+/+		+/+	−/−	–	−/−	–
FAC-004d	Lewers et al. (2005)	+/+		+/+	−/−	–	−/−	–
Fvi9	Ashley et al. (2003)	+/+		+/+	−/−	–	−/−	–
Fvi20	Ashley et al. (2003)	+/+		+/+	−/−	–	−/−	–
UDF002	Cipriani and Testolin (2004)	+/+		+/+	−/−	–	−/−	–
UDF004	Cipriani and Testolin (2004)	+/+	125–154	+/+	±	150	−/−	–
UDF005	Cipriani and Testolin (2004)	+/+		+/+	−/−	–	−/−	–

* Transferable markers across the *Rosoideae* genera mapped in *Fragaria*

[a] Amplification and polymorphism in *F.* × *ananassa* (*F.* × *a.*) cultivars

[b] Size range of PCR bands are only shown for *F.* × *a.* cultivars when primer pairs cross-amplified in *Rosa* or *Rubus*

[c] Amplification and polymorphism in the parental lines of the diploid *Fragaria* reference segregating population. When a primer pair amplified a product in any genotype, a '+' sign is indicated. If a primer pair also detected polymorphisms a '+/+' sign is indicated

[d] Amplification and polymorphism in *Rosa wichurana* (E15) and Blush Noisette (D10)

[e] Amplification and polymorphism in *Rubus idaeus* cultivars Tulameen (Rt) and Miraz (Rm)

evaluated for cross-amplification in two other important genera of the subfamily Rosoideae, rose and raspberry. In order to gain insight into their polymorphism, two species of *Rosa* (Blush Noisette and *Rosa wichurana*) and two accessions of raspberry (*Rubus idaeus* Tulameen, from Canada, and Miraz, from Russia) were analyzed. Cross-amplification in rose and raspberry, allele sizes and the presence/absence of polymorphism are shown in Tables 2–4.

Forty-seven of the 174 SSRs (27%) amplified a product or products of the approximate size expected for a homologous locus in the genus *Rosa* (summarized in Table 5). The transferability of *Fragaria* EST-SSRs was found to be significantly higher than that for genomic-SSRs (28.7 vs. 19.4%), in agreement with its location in more conserved regions of the genome. As expected, due to their phylogenetic relationship (Potter et al. 2007), a lower transferability was obtained for *Rubus,* as only 34 of the SSRs (19.5%) amplified in at least one of the accessions (Table 5). Once more, transferability of EST-SSRs (20.3%) was higher than that of genomic-SSRs (16.1%). The level of polymorphism of *Fragaria* SSRs in *Rosa* and *Rubus* was high even though only two accessions were analyzed for each genus, with 63.8 and 41.2% polymorphic SSRs for rose and raspberry, respectively (Table 5).

Among the *Fragaria* transferable SSRs, 33 markers were polymorphic in the *Fragaria* reference population, in octoploid strawberry cultivars and were transferable to rose. In contrast, only 16 *Fragaria* SSRs were found to be polymorphic in the *Fragaria* reference population, in *F.* × *ananassa* and were also transferable to raspberry. These markers may provide anchor points for genome comparisons between *Fragaria* and these two genera, provided they are also polymorphic in available rose or raspberry mapping populations. Of all the 174 markers analyzed, 14 mapped SSRs cross-amplified in both *Rose* and *Rubus* (denoted in Tables 2–4). Because they are distributed across the seven linkage groups of the *Fragaria* map (Fig. 1 and Sargent et al. 2007), they represent anchor markers for genome comparisons among the three Rosoideae genera.

Discussion

The main goal of this study was to increase the number of functional SSR markers developed in *Fragaria*. About 190 non-duplicated SSR markers

Table 5 Transferability of *Fragaria* SSR markers to *Rosa* and *Rubus*

Type of markers	Number of markers	Amplification	%	Polymorphism	%
Cross-amplification *Fragaria–Rosa*					
EST-SSRs	143	41	28.7	27	65.9
Genomic-SSRs	31	6	19.4	3	50.0
Total	174	47	27.0	30	63.8
Cross-amplification *Fragaria–Rubus*					
EST-SSRs	143	29	20.3	12	41.4
Genomic-SSRs	31	5	16.1	2	40.0
Total	174	34	19.5	14	41.2

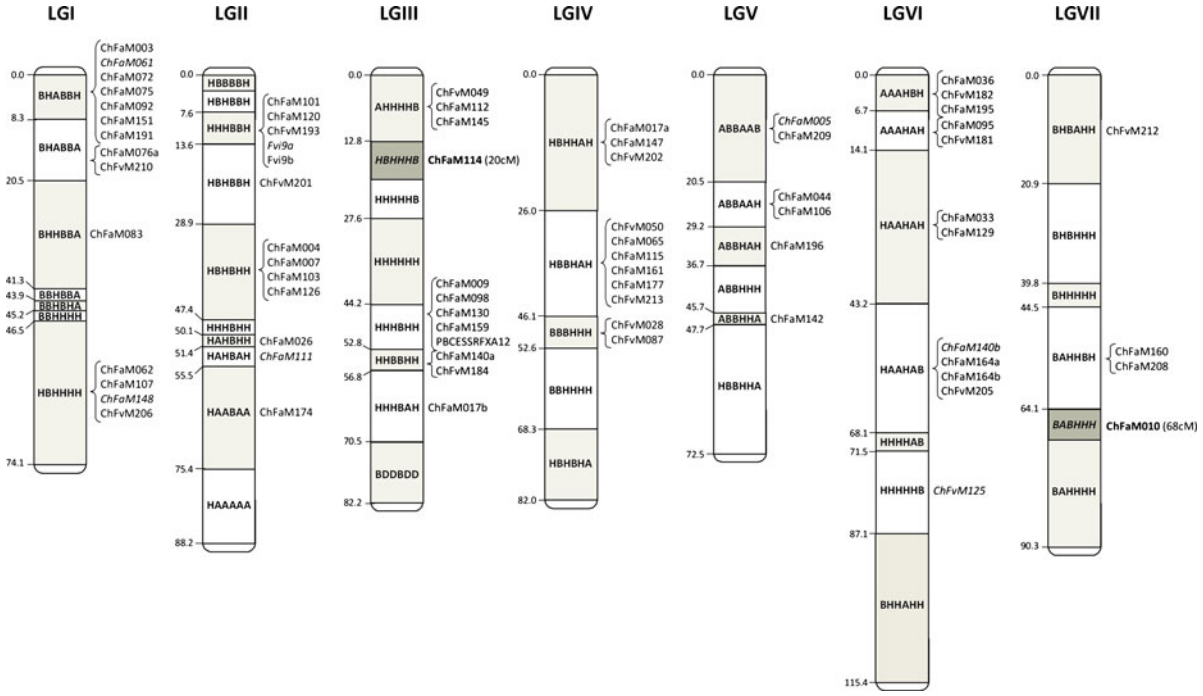

Fig. 1 Bin map of diploid *Fragaria* showing the position of the new genic-SSR markers. The *vertical bars* represent the seven linkage groups divided into the 49 bins defined by the joint genotype of the selected individuals (shown inside each bin). The two new bins defined in this study are shown in *darker shading* and marker names together with the exact position in the map in *bold*. Dominant markers are depicted in *italics*. Genetic distances are shown on the left, indicating the position of the last marker included in the bin according to the framework map

have been published to date from this species (Bassil et al. 2006a; Gil-Ariza et al. 2006; Keniry et al. 2006; Lewers et al. 2005; Monfort et al. 2006; Rousseau-Gueutin et al. 2008; Sargent et al. 2008). With the novel 100 *F.* × *ananassa* genic-SSRs reported here, we have increased the number of available markers by more than 50%. In addition, we have developed 22 novel genic-SSRs from *F. vesca*, which is one of the diploid ancestors of this species. More than 92% of the new markers were polymorphic among strawberry cultivars even though they originate from the transcribed region of the genome and in ~20% of them the SSR was located in the ORF. It has been reported that markers derived from transcribed sequences may be less polymorphic but more robust compared with genomic-SSRs (Varshney et al. 2005). The genic-SSR markers developed here appear to be as polymorphic as genomic-SSRs based on the

number of alleles identified, thus representing a valuable tool for genetic characterization of strawberry.

We found comparable levels of polymorphism between di- and trinucleotide motifs among the strawberry genic-SSRs. Although dinucleotide SSRs usually have higher levels of polymorphism compared to trinucleotide, Fernandez-Silva et al. (2008) showed also similar levels of polymorphism for melon EST-SSRs. It has been extensively reported that the degree of polymorphism increases with the length of the SSR. Here, we also found a correlation between repeat number and polymorphism in the strawberry genic-SSRs. However, this correlation was not observed for the dinucleotide repeats analyzed separately. Other studies have also found no consistent relationship between number of alleles and number of repeats, either across all motifs or individually, and it may become more variable for an intermediate level of repeats (Ferguson et al. 2004; Fernandez-Silva et al. 2008), although the cause for this remains elusive.

Based on homology analyses, functions were assigned for more than 67% of the ESTs from where the markers were developed, whereas a further 10% were derived from sequences with similarity to unknown proteins. About 23% of the ESTs did not contain significant similarity to any protein, either because of the limited sequence available or because they are specific to strawberry. Since all of them can be considered functional markers, they are candidates for quantitative trait loci and can be associated to phenotypic or adaptive variation of strawberry fruit. Examples of this are the markers ChFaM079, ChFaM126 and ChFvM210, which may be related to hormonal responses during fruit growth and ripening, as they show homology to proteins induced by auxin or abscisic acid (Tables 2 and 3).

All SSRs derived from *F. vesca* cross-amplified in *F.* × *ananassa* and therefore their transferability was 100%. Other studies have also shown high transferability levels between *F. vesca* and *F.* × *ananassa,* ranging from 94 to 100% (Cipriani and Testolin 2004; Hadonou et al. 2004; Monfort et al. 2006). These results are in agreement with an ancestor of *F. vesca* contributing to the genome of *F.* × *ananassa* (Rousseau-Gueutin et al. 2009). In general, it has been reported that rapid genomic changes occur after polyploidization (Chen 2007). The most frequent modification is the loss of homoeologous copies of many duplicated genes, retaining at least one copy in one homoeologue genome. However, such a high level of cross-amplification of *F. vesca* sequences suggests that these changes have not been dramatic in *F.* × *ananassa*. This is supported by the high colinearity recently found between these genomes, indicating that major rearrangements have not occurred (Rousseau-Gueutin et al. 2008; Sargent et al. 2009a). The transferability of *F.* × *ananassa* SSRs to *F. vesca* was only 89.1%, similar to the level of cross-amplification (89%) reported by Bassil et al. (2006a). The failure of SSRs derived from *F.* × *ananassa* to amplify in *F. vesca* may be due to divergence in those sequences between the species. This appears unlikely since a similar cross-amplification level would be expected for the *F. vesca* SSRs in *F.* × *ananassa*. A more plausible explanation is that these markers specifically amplify strawberry homoeologues contributed by another donor after hybridization. Interestingly, out of the five strawberry SSRs failing to amplify in all *F. vesca* accessions tested, two markers (ChFaM047 and ChFaM070) amplified fragments of the expected size in *F. iinumae*. Two other markers (ChFaM022 and ChFaM069) were specific to some *F. vesca* accessions. Ongoing mapping studies in *F.* × *ananassa* may reveal whether these SSRs are specific markers for the Z and Y1 genomic pools of the octoploid *Fragaria* genome.

The bin mapping of 72 genic-SSR markers has significantly increased the overall number of markers in the diploid *Fragaria* reference map to about 420 molecular loci (Sargent et al. 2008, 2009b). The SSRs mapped in this study were, in general, assigned to defined FV × FB bins. However, two markers, ChFaM010 and ChFaM114, defined new bins in linkage groups VII:68 and III:20 respectively, covering regions with a reduced density of markers. Additionally, a number of SSRs mapped in this study were localized to bins with medium to low marker density, significantly increasing map saturation. The use of this bin mapping strategy allows the efficient and cost-effective localization of markers in genetic maps, which in turn may help in the validation of candidate genes located in the vicinity of qualitative or quantitative traits. As an example, the marker ChFvM125, identified in the fosmid EU024861, is located at about 10 kb distance from *FaCO*, a homologue of the *Arabidopsis* gene *CONSTANS*

(*CO*) which participates in the photoperiodic control of floral induction (Putterill et al. 1995). The *SEASONAL FLOWERING LOCUS* (*SFL*) regulates seasonal flowering in the wild diploid *F. vesca*, which flowers after short day induction. The position of *SFL* has been reported at 29.6 cM on linkage group VI (Albani et al. 2004; Cekic et al. 2001; Sargent et al. 2004), indicating that *FaCO* is not the gene responsible for the seasonal flowering in *F. vesca*. Similarly, our results also excluded a *TFL1*-like gene (marker ChFaM142), mapped in bin V:48, as being the *SFL* locus (Fig. 1).

Many studies have addressed the cross-amplification of SSR markers within families. As examples, a transferability of about 35% has been reported from tomato to pepper (Frary et al. 2005) and up to 60% among cereals such as rice, wheat, barley and maize (Gupta et al. 2003; Tang et al. 2006). The transferability within the Rosaceae family has been shown to be lower (Decroocq et al. 2003; Gasic et al. 2009). Recently, a close phylogenetic relationship between *Fragaria* and *Rosa* has been reported while the genus *Rubus*, also belonging to the Rosodae supertribe, is not as closely related (Potter et al. 2007). In accordance with their phylogenetic distances, the transferability of *Fragaria* SSRs was 27 and 19.5% to *Rosa* and *Rubus*, respectively. This study has also shown that cross-amplification of genic-SSRs was significantly higher than that of genomic-SSRs: 28.7 vs. 19.4% for *Rosa* and 20.3 vs. 16.1% for *Rubus*. Transferability of *Malus* EST-SSRs to *Pyrus*, *Fragaria*, *Rosa* and *Prunus* has been reported recently (Gasic et al. 2009). The highest transferability, 62%, was found to the closely related pear, followed by *Prunus*, with 56%. Although *Malus* is distantly related to *Fragaria* and *Rose*, transferability to these genera was high, with 48 and 28%, respectively. In accordance with our results, low rates of SSR cross-amplification have been reported between Rosoideae genera, with transferability rates of *Fragaria* SSRs to *Rubus* of between 27 to 19% (Lewers et al. 2005). In contrast, these authors found that none of the 30 *Rosa*-derived SSRs amplified products in *Fragaria* or *Rubus*. In a recent report, a low transferability of genomic SSRs from peach and apple to rose was found: 17 and 8%, respectively (Oyant et al. 2008). In contrast to the results reported here, cross-amplification of *Fragaria* SSRs in rose was very high (76%), although only 17 SSRs were evaluated. Overall, these results suggest that sequence conservation is higher among members of the Spiraeoideae, such as between *Prunus* and *Malus*, than among the Rosoideae subfamily.

We have found some inconsistencies between our results and previously published data (Lewers et al. 2005). Thus, in the case of marker ARSFL_35, which is located in the cellulase gene *Cel2*, we obtained a clear cross-amplification in both raspberry accessions tested, while it did not amplify in the study of Lewers et al. (2005). This genic-marker might be a valuable tool for genome comparisons since it is polymorphic in all tested genera within the Rosoideae. Also, marker EMFv010 did not amplify any of the tested raspberry accessions in the study of Lewers et al. (2005) but amplified one accession in our study, whereas marker Fvi20 cross-amplified in their work but not in ours. These differences might be due to the different accessions tested, besides the wide diversity reported within the *Rubus* genera (Jennings 1988). Finally, an important issue in cross-amplification evaluation is the PCR conditions used, which have been maintained quite stringently in our study. Lowering the T_a in the reaction may increase transferability, but false positives can appear and will produce incorrectly scored loci and errors in genome comparisons.

Besides cross-amplification, it is necessary that a marker developed for one species can detect polymorphism at homologous loci in related genera. Despite other markers such as conserved orthologous sequences that have been shown to be more transferable across taxa, SSRs are characterized by a high level of polymorphism. In this study, we have shown a high level of polymorphism of *Fragaria* SSRs in both *Rosa* and *Rubus*, even though only two accessions were evaluated for each genus. Therefore, although SSR markers are less transferable across taxa, the probability of polymorphism between mapping genotypes is expected to be higher. Furthermore, since genic-SSRs are derived from transcribed genes, they are more conserved and transferable than anonymous sequence-derived markers. Our study has shown that genic-SSRs are significantly more transferable than SSRs obtained from anonymous genomic sequences.

Acknowledgments This work was supported by grants RTA2008-00029-00-00 (INIA, partly funded by FEDER, European Union), AGR-03230 (CICE, Junta de Andalucía)

and BIO2007-67509-C02-01 (MEC). The IFAPA *Fragaria* germplasm collection is funded by grant RFP2008-00009-00-00 (INIA). At CRAG, this work was supported with funds of the Spanish Ministry of Science and Innovation, project RTA2007-00063-00-00 (INIA). Y.Z-F. was supported by a fellowship and I.A. by a contract from INIA. We are gratefull to E. Cruz-Rus for obtaining the genomic sequence of GaLUR.

Ethical standards The experiments of this study comply with the current laws of Spain.

Conflict of interest The authors declare that they have no conflict of interest.

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